Supporting Information

Effect of Molecular Crowding and Ionic Strength on the Isothermal Hybridization of Oligonucleotides

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For each oligonucleotide strand length we wished to perform isothermal experiments at temperatures where parameters such as crowding and ionic strength would have the greatest chance to induce pairing. Thus, melting profiles for each strand were recorded to find the minimum temperature for "complete" melting, Figure S1. The experiments were carried out on a CARY-100 spectrophotometer; absorbance changes at 260 nm were recorded with increasing temperature. Initially, the complimentary oligomers were paired at 50 mM NaCl and stored at 4 ^{o}C for an hour. The samples were then diluted with 1 mM phosphate buffer to a final strand concentration of 2 μM and Na^{+} of 2.5 mM. Melting profiles were collected between 5 ^{o}C or 20 ^{o}C (for the 15-mer and the 25-mer and 35-mer strands respectively) and 90 ^{o}C at 0.3 ^{o}C/min, while the sample chamber was being purged with N_2 to eliminate condensation of water on the cells at low temperatures. Melting temperatures, T_m, were determined using the Thermal software for spectral collection and analysis (Varian, Inc.) and the desired temperatures were selected in a way to ensure the strands at the given conditions are in the single stranded form.

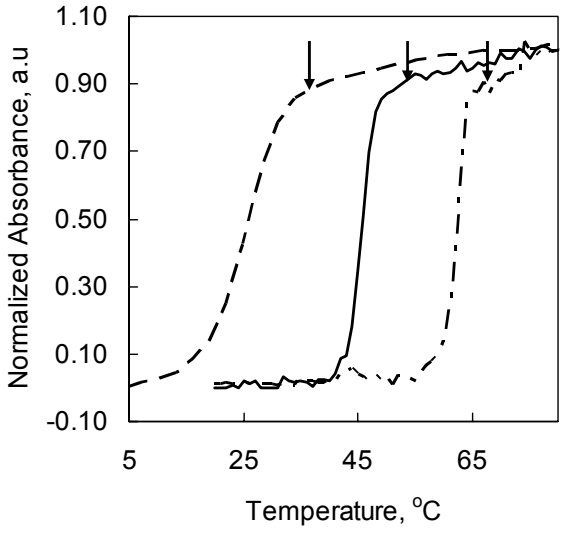

Figure S1: Melting profiles of the 15-mer (– – -), 25-mer (——), and 35-mer (– · –) oligonucleotides at 2 μM total single strand concentration and 2.5 mM Na^+ concentration. The arrows indicate the temperature at which the isothermal experiments were performed; 35 °C, 55 °C, and 67 °C for the 15-mer, 25-mer and 35-mer strands respectively.

Table S1: Summary of Na^+ required for the hybridization of the oligomers at different PEG3 concentrations.

		[Na^+], mM pairing range	[Na^+], mM @ 50% pairing			
	%GC	0% PEG3	0% PEG3	1% PEG3	10% PEG3	30% PEG3
15-mer @ 35 °C	33	10 - 135	39	35	25	16
25-mer @ 55 °C	32	35 - 222	70	65	50	29
35-mer @ 67 °C	51	25 - 115	40	31	27	14

Osmometry was performed on a Vapro 5520 Vapor Pressure Osmometer by Wescor, Inc. at 20 °C. Figure S2 represents the data points collected at different PEG 4000 (average molecular weight 3000) (PEG3)/NaCl combinations in phosphate buffer at 1 mM and pH 7.0.

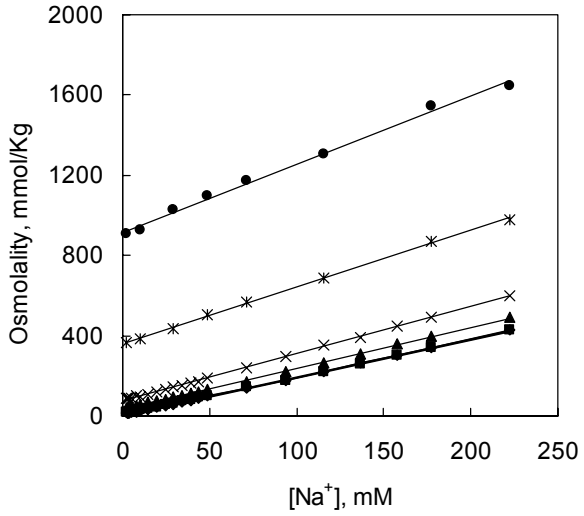

Figure S2: Osmolalities of PEG3/NaCl/buffer solutions measured by vapor pressure osmometry at 20 °C and at 0 wt% PEG3 (♦), 1 wt% PEG3 (■), 5 wt% PEG3 (▲), 10 wt% PEG3 (×), 20 wt% PEG3 (*), and 30 wt% PEG3 (●).

Table S2: Line fits to the data sets of increasing osmolality with increasing NaCl concentration presented in Figure S2.

PEG3 content, wt%	Line fit	R^2 value
0	y = 1.8867x + 5.0320	0.9991
1	y = 1.8380x + 11.956	0.9994
5	y = 2.0444x + 34.132	0.9993
10	y = 2.3387x + 76.266	0.9995
20	y = 2.8217x + 360.30	0.9993
30	y = 3.3948x + 917.18	0.9946

From the osmometry data, the activity of water in the crowded solutions was determined using the following equation:[1]

$$\ln a_w = -10^{-6} M_1 (mOsm)$$

where a_w is the activity of water, M_1 is the molecular weight of water and *mOsm* is the milliosmolality of the solution.

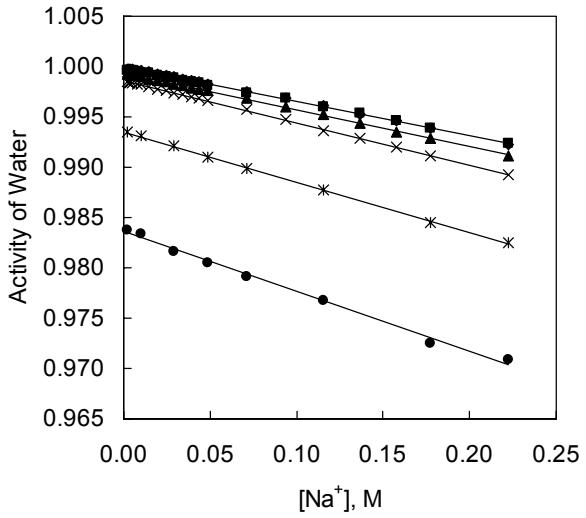

Figure S3: Activity of water with increasing NaCl concentration in PEG3/buffer solutions determined from vapor pressure osmometry at 20 °C and at 0 wt% PEG3 (♦), 1 wt% PEG3 (■), 5 wt% PEG3 (▲), 10 wt% PEG3 (×), 20 wt% PEG3 (*), and 30 wt% PEG3 (●).

Table S3: Line fits to the data sets of water activity versus increasing NaCl concentration at different PEG3 wt% presented in Figure S3.

PEG3 content, wt%	Line fit	R^2 value
0	y = -0.0338x + 0.9999	0.9992
1	y = -0.0330x + 0.9998	0.9994
5	y = -0.0366x + 0.9994	0.9994
10	y = -0.0419x + 0.9986	0.9995

20	y = -0.0502x + 0.9935	0.9993
30	y = -0.0597x + 0.9836	0.9945

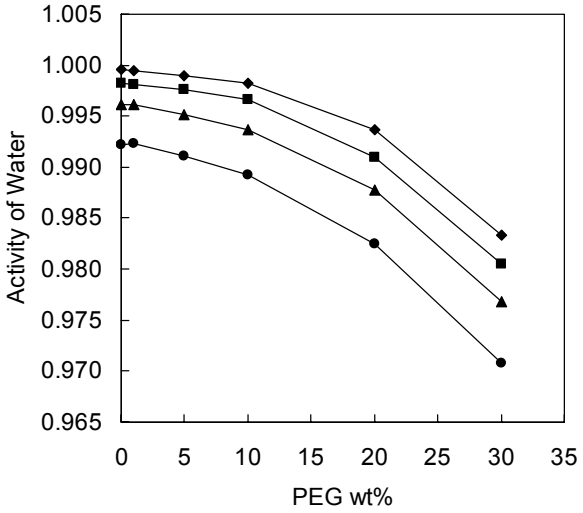

Figure S4: Activity of water versus increasing PEG3 wt% in phosphate buffer @ 1mM buffer capacity and pH 7.0 and different Na^+ concentrations: 9.94 mM (♦), 48.4 mM (■), 115.8 mM (▲), and 222.5 mM (●). Lines are guides to the eye.

Next we determined the activities of sodium ions from the osmolalites of solutions determined by vapor pressure osmometry. In a recent study, Capuano et al.[2] reported the obstructive properties of PEG in NaCl/mixed-solvent (PEG-water) solutions where the molar conductance of the NaCl solutions was shown to be independent of PEG molecular weight when correcting for the PEG volume fraction in the solutions, in the absence of electrostatic interactions between PEG chains and sodium ions.[3] Similarly, we considered the osmolalities of the solutions to be the additive contribution of the individual osmolalities of the different solutes.

$$[mOsm]_{total} = [mOsm]_{PEG} + [mOsm]_{NaCl} + [mOsm]_{buffer}$$

$$[mOsm]_{NaCl} = [mOsm]_{Na^+} + [mOsm]_{Cl^-}$$

$$a_{Na^+} = [mOsm]_{Na^+} \times \rho$$

Here we assume

$$[mOsm]_{Na^+} = [mOsm]_{Cl^-}$$

As a result the activities of sodium ions can be written as follows:

$$a_{Na^+} = \frac{[mOsm]_{NaCl}}{2} \times \rho$$

Where the individual milliosmolalities, [*mOsm*], are indicated by the appropriate subscript; a_{Na^+} stands for the activity of sodium ions and ρ for the density of the solution. ρ was determined from consecutive weighings of 500 μL samples of each solution.

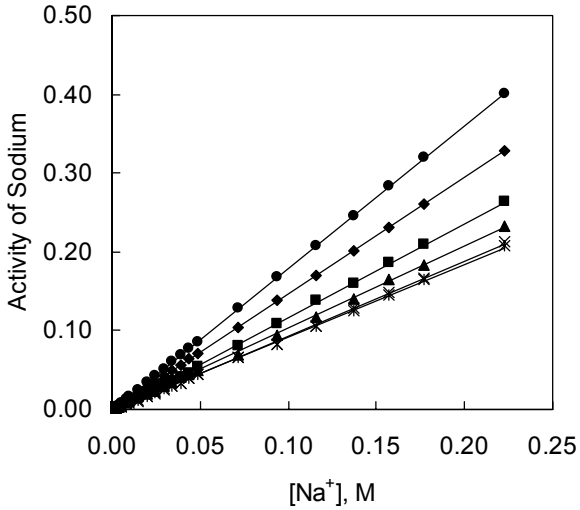

Figure S5: Activity of sodium ions at different NaCl concentration in different PEG3/buffer solutions; 0 wt% PEG3 (*), 1 wt% PEG3 (×), 5 wt% PEG3 (▲), 10 wt% PEG3 (■), 20 wt% PEG3 (♦), and 30 wt% PEG3 (●). For concentrated PEG, the activity of sodium is greater than its concentration due to the volume occupied by PEG.

Table S4: Line fits to the activity of sodium ions versus increasing NaCl concentration at different PEG3 wt% presented in Figure S5. The intercept of all lines was set to 0.

PEG3 content, wt%	Line fit	R^2 value
0	y = 0.9364x	0.9986
1	y = 0.9203x	0.9993
5	y = 1.0338x	0.9993
10	y = 1.1733x	0.9987
20	y = 1.4697x	0.9999
30	y = 1.8006x	0.9999

Next we present the activity of water versus the increasing activity of sodium ions in different PEG3 solutions, where the slope of the fits shows a comparable decrease in water activity with increasing Na^+ activity regardless of crowding conditions, unlike the slopes in Figure S3.

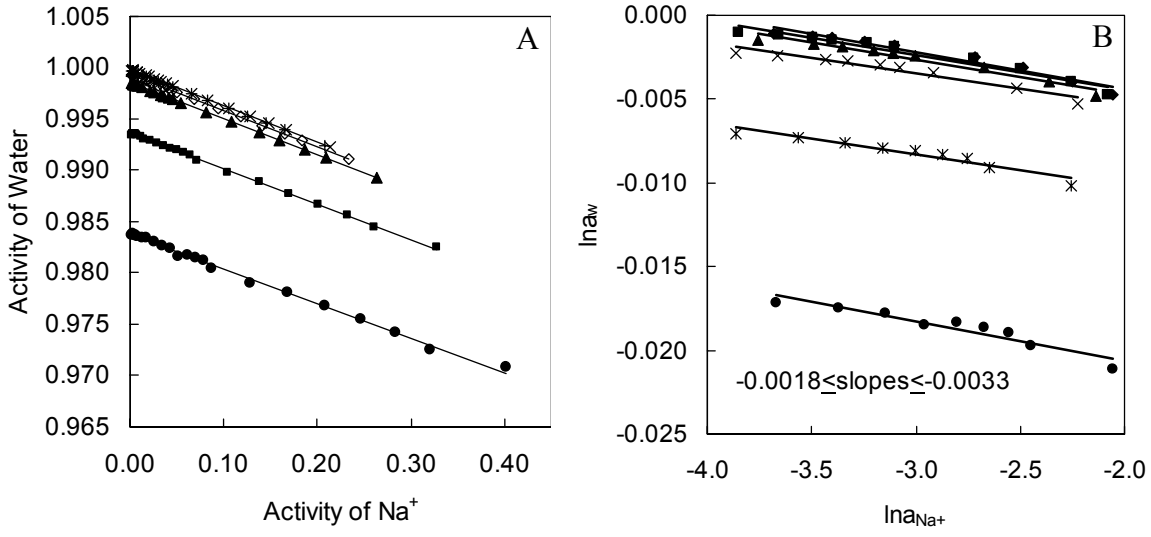

Figure S6: Panel A: Activity of water with increasing activity of Na^+ in PEG3/buffer solutions determined from vapor pressure osmometry at 20 ^oC and at 0 wt% PEG3 (×), 1 wt% PEG3 (+), 5 wt% PEG3 (◊), 10 wt% PEG3 (▲), 20 wt% PEG3 (■), and 30 wt% PEG3 (●). Panel B: $\ln a_w$ versus $\ln a_{Na+}$ in the working range of a_{Na+} where the pairing transition is observed; lines represent fits to the data points and inset shows the range of slopes used in Equation 4 to correct for the sodium ions bound to the oligonucleotides.

Table S5: Line fits to the data sets of water activity versus increasing Na^+ activity at different PEG3 wt% presented in Figure S6. After correction for sodium activity, the increase in water activity with increasing NaCl in solution is the same for all solutions at different PEG3 wt%.

PEG3 content, wt%	Line fit	R^2 value
0	y = -0.0355x + 0.9998	1
1	y = -0.0355x + 0.9997	1
5	y = -0.0353x + 0.9994	1
10	y = -0.0350x + 0.9985	1
20	y = -0.0346x + 0.9936	0.9993
30	y = -0.0337x + 0.9838	0.9966

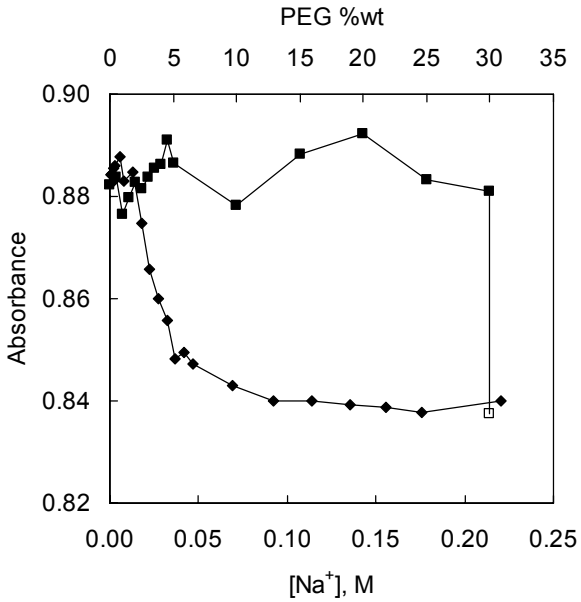

Figure S7: Hypochromicity of beacon at 260 nm and 75 ^oC (♦) with increasing concentration of Na^+ ions (primary x-axis) and increasing wt% of PEG3 (■) (secondary x-axis). The open square indicates the addition of Na^+ to the DNA solution in PEG/buffer

to a final concentration of 0.125 M. This "hairpin" experiment starts closer to the melting temperature, thus, a lower [NaCl] is required for pairing.

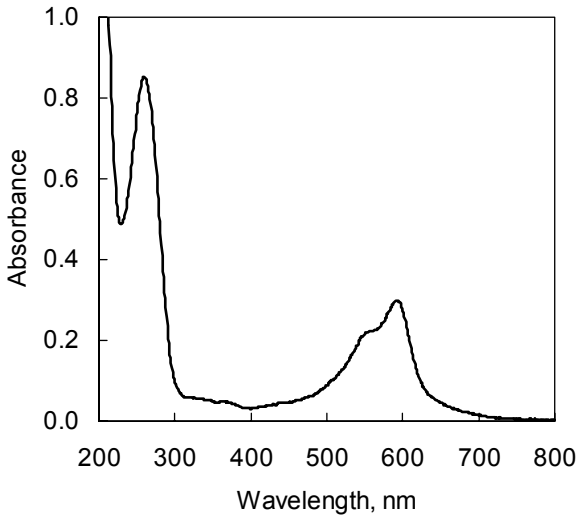

Figure S8: UV-vis spectrum of the hairpin beacon at 75 ^{o}C in 1 mM phosphate buffer at pH 7.0 containing 27.5 mM Na^{+}. Three absorbance peaks are observed, @ 260 nm corresponding to the DNA bases, and @ 555 nm and 590 nm corresponding to the Texas Red and Black Hole Quencher-2, dye quencher pair.

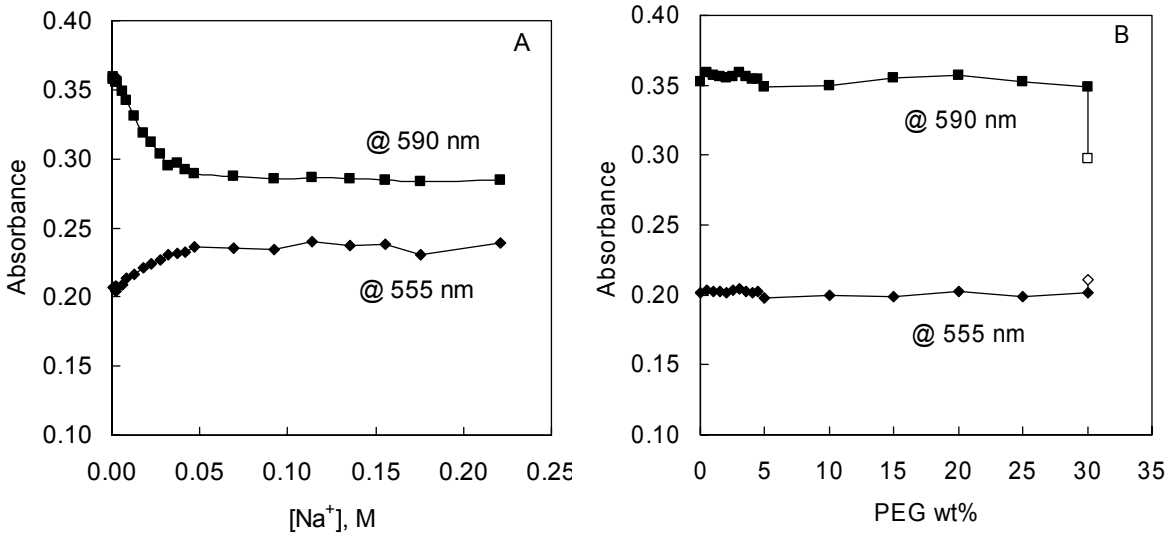

Figure S9: Absorbance change of the dye quencher pair of the beacon at 75 ^{o}C at 555 nm and 590 nm with increasing [NaCl] in solution (panel A) and PEG3 wt% (panel B). In panel A, The hairpin starts associating at lower salt concentrations than the free oligomers because the experiment starts closer to the melting temperature. Open points in

panel B correspond to the change of absorbance as NaCl was added to the solution to a final concentration of 0.125 M.

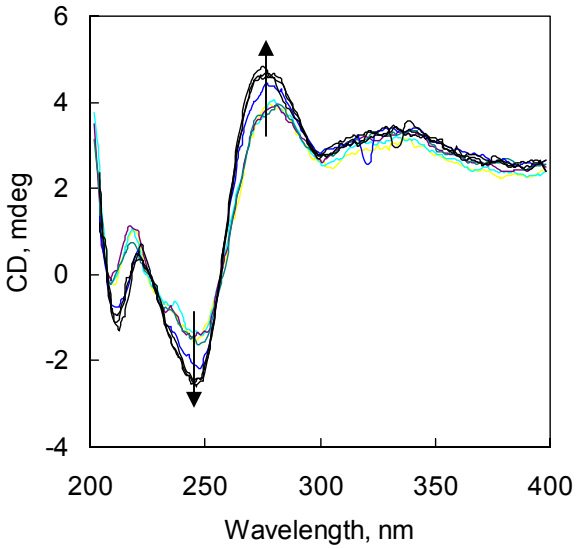

Figure S10: Circular dichroism spectra of the complimentary 35-mer oligonucleotides in 1 mM phosphate buffer at pH 7.0 and 67 ^{o}C with increasing NaCl concentration. The arrows indicate the change in peak intensity with increasing [Na^{+}] which levels off after pairing is complete. The spectra of the paired DNA correspond to that of a B-form double stranded DNA in agreement with the observations published in the literature.[4-5] Each spectrum was performed in triplicate and averaged.

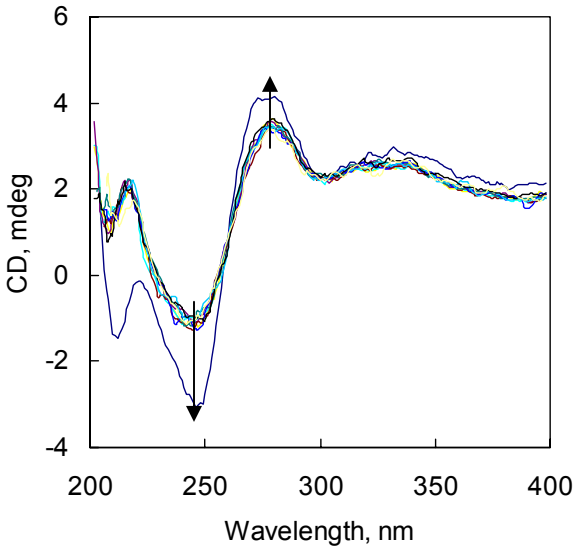

Figure S11: Circular dichroism spectra of the complimentary 35-mer oligonucleotides in 1 mM phosphate buffer at pH 7.0 and 67 ^{o}C with increasing wt% PEG3 up to 30%. The

peaks at 275 nm and 245 nm did not change indicating no pairing and no interaction of the strands with the PEG3 macromolecules. Upon addition of NaCl the peak values changed (arrows) without shifts in wavelength indicating the pairing of the strands. Each spectrum was performed in triplicate and averaged.

In order to ensure our measurements were not kinetically controlled, especially at low salt concentrations, we looked at the pairing of the oligonucleotides at different salt concentrations and temperatures. Figure S12 is a depiction of the behavior of the 35-mer at, respectively, 20 mM (panel A) and 157 mM (panel B) [Na^+] both at 67 ^oC and 20 ^oC. The absorbance signal of the bases was followed at 260 nm before and after addition of the appropriate aliquot of NaCl. Within 10 minutes of addition of the salt, at 20 mM and at 67 ^oC, the signal was at a stable plateau. Then the temperature was decreased to 20 ^oC where the signal decreased and was constant when temperature equilibration was achieved. Next the temperature was increased to 67 ^oC and the increase in absorbance was recorded which was identical to the signal recorded after the addition of the NaCl initially. The same procedure was repeated on a new sample where enough NaCl was added to increase the oligomer Na^+ concentration to 157 mM, after the initial addition and at 67 ^oC, the signal decreased within a few minutes and was constant after five minutes. Following, was a decrease in the solution temperature to 20 ^oC where no change in the signal was observed and finally the temperature was once again increased to 67 ^oC with no change in the absorbance behavior of the strands. The latter indicates the complete pairing of the single strand oligomers both at 67 ^oC and 20 ^oC at 157 mM, whereas at 20 mM the strands are in the 100% paired form at 20 ^oC but are partially paired at 67 ^oC which is not a kinetic hindrance rather a reflection of the equilibrium state of the DNA strand pairing at the given conditions.

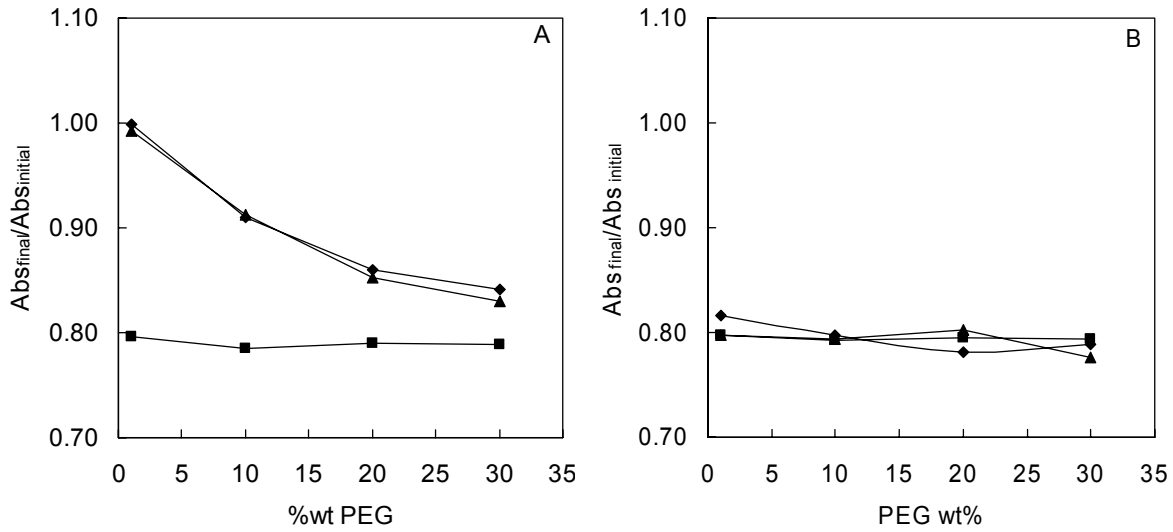

Figure S12: Absorbance signal decrease upon pairing of complimentary 35-mer oligomer strands at 20 mM [Na^+] panel (A) and 157 mM [Na^+] panel (B). In both graphs, (♦) represent the first measurement at 67 ^oC, the (■) are data points for the second measurements at 20 ^oC and (▲) are points for the third absorbance scans at 67 ^oC.

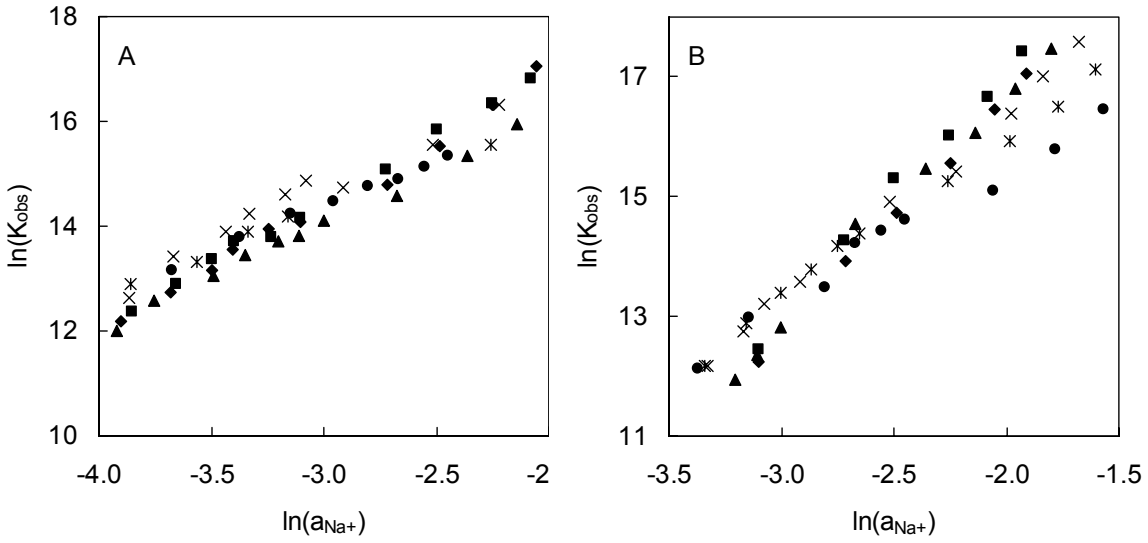

Figure S13: Plots of $\ln K_{obs}$ versus $\ln a_{Na+}$. (A) 15-mer @ 35 ^{o}C (B) 25-mer @ 55 ^{o}C. In both graphs data points are at 0 wt% PEG3 (♦), 1 wt% PEG3 (■), 5 wt% PEG3 (▲), 10 wt% PEG3 (x), 20 wt% PEG3 (*), and 30 wt% PEG3 (●).

Table S6: Number of sodium ions added to a base pair upon hybridization of oligomers with increasing molecular crowding. The change in water molecules per strand observed upon pairing of the oligonucleotides at different sodium ion activities.

	15-mer		25-mer		35-mer	
% PEG3	$-\Delta n_{Na^+}{}^{a}$	$\Delta\psi^{b}$	$-\Delta n_{Na^+}{}^{a}$	$\Delta\psi^{b}$	$-\Delta n_{Na^+}{}^{a}$	$\Delta\psi^{b}$
0	0.18	0.36	0.17	0.34	0.09	0.18
1	0.17	0.34	0.17	0.34	0.09	0.18
5	0.14	0.28	0.16	0.32	0.08	0.16
10	0.16	0.32	0.13	0.26	0.07	0.14
20	0.12	0.24	0.12	0.24	0.05	0.10
30	0.12	0.24	0.10	0.20	0.04	0.08
$-\Delta n_w$ @ $a_{Na^+} = 0.02$	-14		-34		-62	
$-\Delta n_w$ @ $a_{Na^+} = 0.05$	8		10		6	
$-\Delta n_w$ @ $a_{Na^+} = 0.40$	68		114		165	

[a] Columns $-\Delta n_{Na^+}$ represent the values obtained from the slopes of $\ln K_{obs}$ vs. $\ln a_{Na+}$. [b] Columns $\Delta\psi$ represent the number of bound ions per base pair when considering the non-ideality of the DNA strands, see Reference 6.

References

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